Applied PhytoGenetics, Inc.

- APGEN, Inc. is a phytoremediation company.
- Phytoremediation is using plants to clean polluted soil and water
  - Degrade organics
  - Detoxify and sequester toxic elements like heavy metals

Scientific Team

- Richard B. Meagher, Ph.D. - Chief Scientific Officer
- Laura Carreira, Ph.D. - Principal Research Officer

APGEN is based in Georgia

- APGEN is located in University of Georgia's Biotechnology Development space
  - Rm 169, 110 Riverbend Rd.
  - Athens, GA 30602-7223
  - (706) 546-4238
- APGEN has been partially supported by the Georgia Research Alliance and has proprietary and patented technologies from UGA

Why use phytoremediation?

- Plants are photosynthetic and have 80% of the energy in most ecosystems (the dominant organisms)
- Plants have extensive root systems for mining nutrients or pollutants (grow 100 million miles/acre/year).

Why use phytoremediation?

- Remarkably effective.
- Cost effective - 1/10th the expense of physical methods (capping, excavation, and reburial, soil roasting).
- Permanent solution as compared to reburial or capping of hazardous waste.
- Green solutions improve the environment and have strong public support.

How does phytoremediation work?

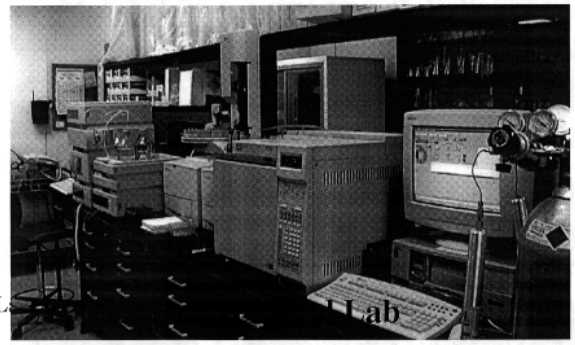
- Organic pollutants are degraded to harmless small molecules (win-win).
- Elemental pollutants are detoxified, sequestered, and may be hyperaccumulated for harvest.

APGEN's Approach to the Phytoremediation of Toxic Organics

- Treatability studies find particular plant species that degrade organic contaminants
- Plants selected from many native species environmentally suited to the site
- Field test the plants and clean up the site

APGEN's treatability studies identify those plant species that degrade organic pollutants in sediment or water samples from a site.

APGEN's analytical methods quantify the degradation of pollutants by plants.



L Lab

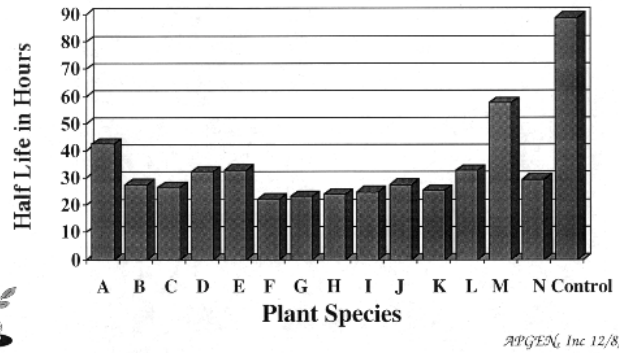
We have quantitative data on the phytodegradation of organics such as:

- Chlorinated solvents
- Benzopyrenes
- Poly-Aromatic Hydrocarbons (PAHs)
- Phthalates
- Benzene
- DDD
- DDT

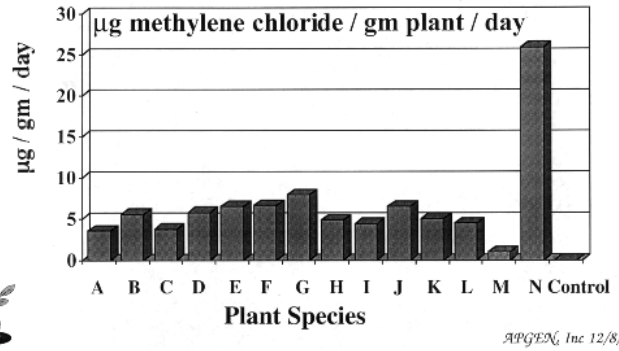
Plant Selection for Solvent Waste Disposal Site

- 68 rapidly growing plant species suitable to the site were initially analyzed
- 14 species significantly accelerated the rate of degradation for one or more target compounds (methylene chloride, chloroform, benzene)

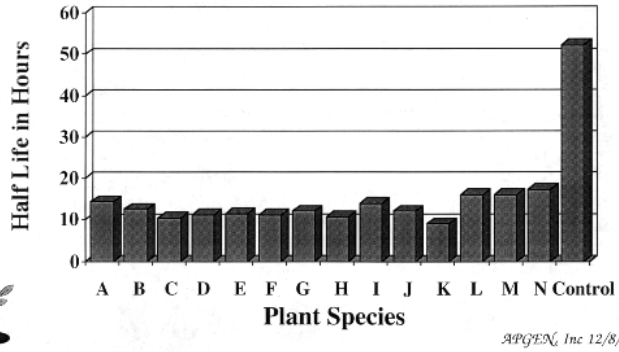
Selected plant species shorten the half-life of methylene chloride



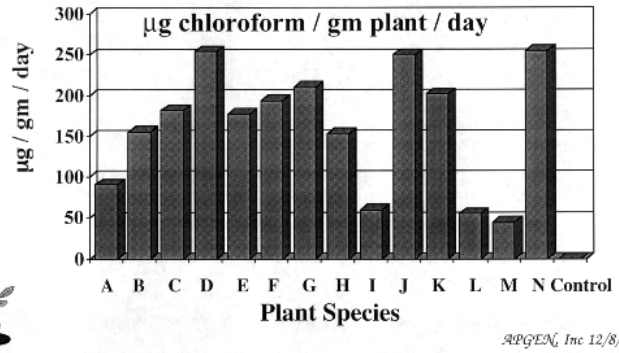
Specific activities for methylene chloride removal determine plant biomass needed



Selected plant species shorten the half-life of chloroform



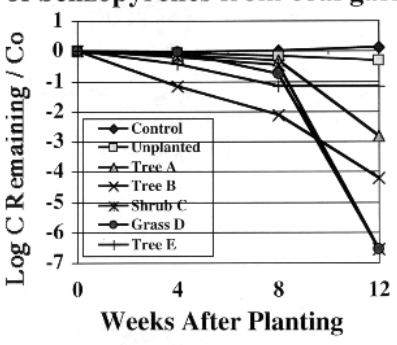
Specific activities of plants for chloroform removal determine biomass needed



Compact willow accelerates the rate of degradation for all three target compounds

Red bay accelerates the rate of degradation for methylene chloride and chloroform, but not benzene

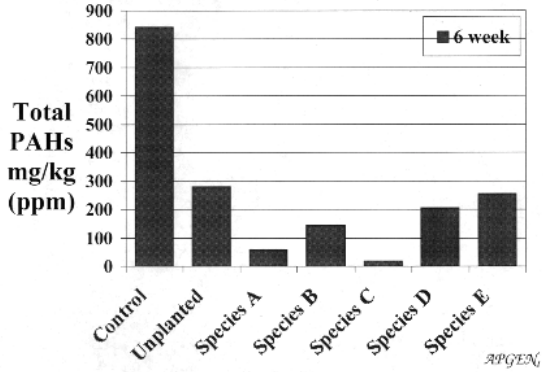
Selected plants dramatically enhance degradation of benzopyrenes from coal gassification site



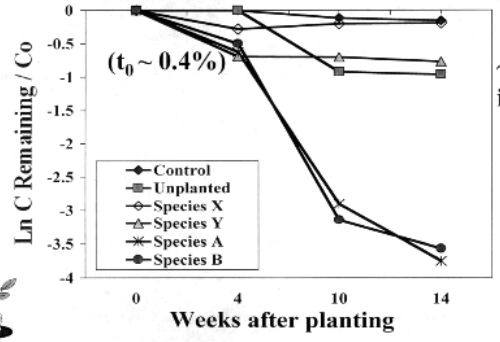
30 species this range

3 species this range

Plants enhance degradation of Poly-Aromatic Hydrocarbons (PAHs) in coke oven soils



Selected plants degrade extractable PAHs and phthalates in soil #1 at floor tile plant



~23 Species in this range

12 Species in this range

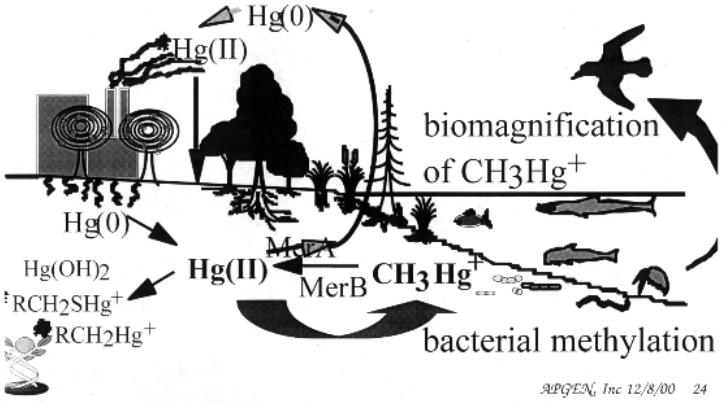
Toxic elemental pollutants (e.g., heavy metals, radionucleides) present special problems

- **Elemental pollutants are immutable short of nuclear fission or fusion**
- **Phytoremediation goals are detoxification, sequestration, extraction, and/or hyperaccumulation**

APGEN's Approach to Toxic Elemental Pollutants

- **Test assertive genes in model plant species for enhancement of detoxification and/or accumulation**
- **Move genes into native conservation plant species suited to a variety of sites**

Bio-geochemical of mercury



"Mercury species are subject to much faster atmospheric removal than elemental mercury"

Keating *et al.*, 1997
EPA's "Mercury Study Report to Congress"

Conclusion: Hg(0) will be globally diluted into an ancient pool of mercury

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Conclusion from report: Hg(0) will be globally diluted into an ancient pool of mercury

Methylmercury is a more serious problem than ionic mercury.

- Methylmercury is more toxic
- Methylmercury is very efficiently biomagnified up the food chain up to 10^8 - fold.
- Methylmercury is produced at all aquatic mercury sites

Methylmercury can be broken down to less toxic non-biomagnified products

10^6-10^8 Biomagnified		Not biomagnified		Not biomagnified
CH_3Hg^+	MerB →	Hg(II)	MerA →	Hg(0)↑
methyl-mercury		ionic mercury		metallic mercury

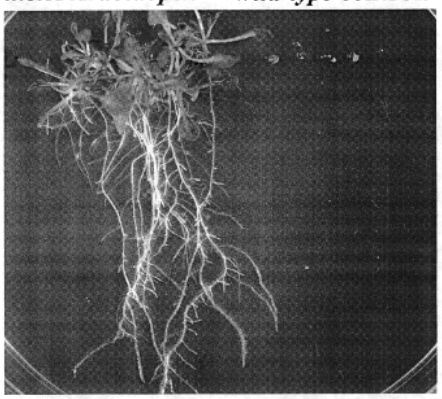
First consider the MerA catalyzed reduction of ionic to less toxic metallic mercury

Hg(II) $\xrightarrow{\text{MerA}}$ Hg(0)↑

Ionic mercury Metallic mercury

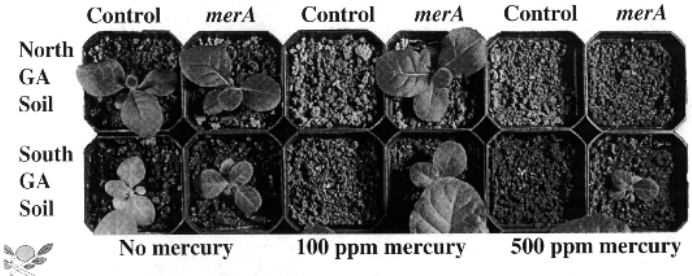
Plants expressing the bacterial *merA* gene are resistant to levels of mercuric ion that kill controls.

merA Arabidopsis *wild-type* controls

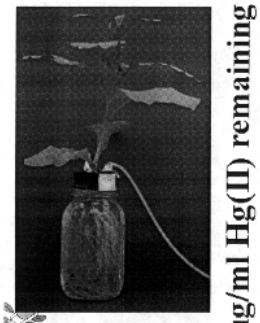


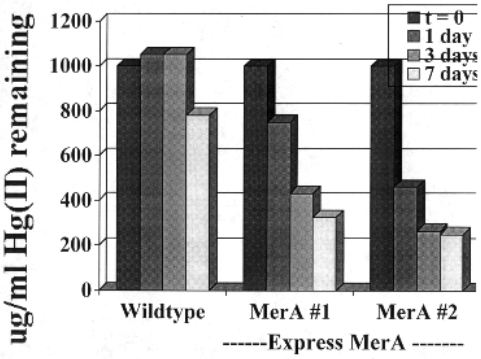
50 μM Hg(II)

APGEN's patented *merA* plants resist and detoxify mercury in polluted soils.

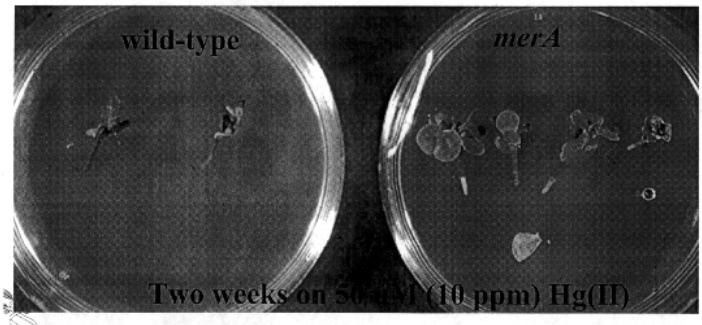


APGEN's plants will efficiently remove mercury from liquid industrial waste



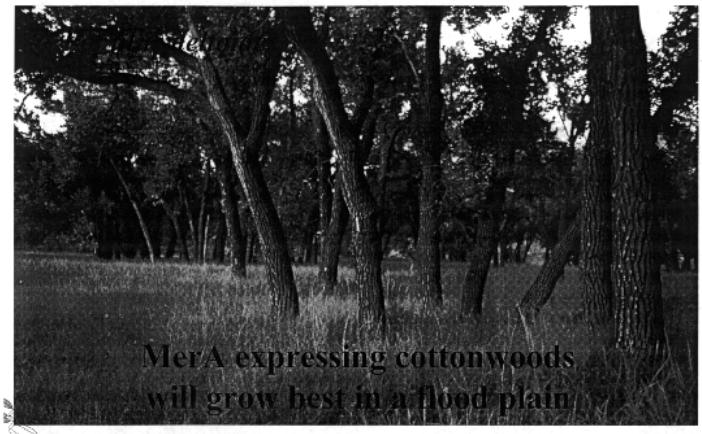


merA transgenic yellow poplar plantlets are resistant to Hg(II), while the controls are not



Liriodendron

Rice plants expressing *merA* clean Hg(II) from aquatic sediments



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Minamata Bay, Japan and several other mercury poisoning incidents illustrated the tragic human consequences of methylmercury biomagnification in aquatic ecosystems.

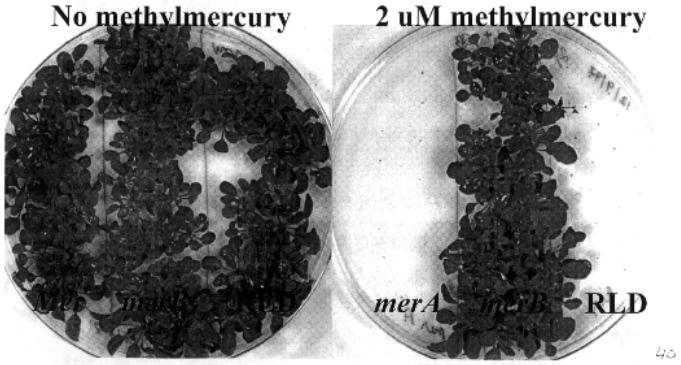
Methylmercury can be broken down to less toxic non-biomagnified products

10^6-10^8 Biomagnified		Not biomagnified		Not biomagnified
CH_3Hg^+	$\xrightarrow{\text{MerB}}$	Hg(II)	$\xrightarrow{\text{MerA}}$	Hg(0)↑

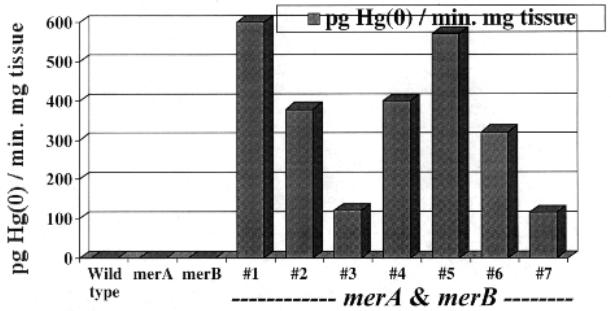
APGEN's plants can handle methylmercury (MeHg) in several ways

- Detoxify MeHg and accumulate Hg(II) below ground
- Detoxify MeHg and eliminate mercury from site as Hg(0)
- Current research on detoxifying MeHg and hyperaccumulating Hg(II) above ground

Transgenic *merB* expression in *Arabidopsis* degrade methylmercury and accumulate Hg(II)



merA & *merB* are both required to convert methylmercury to Hg(0)



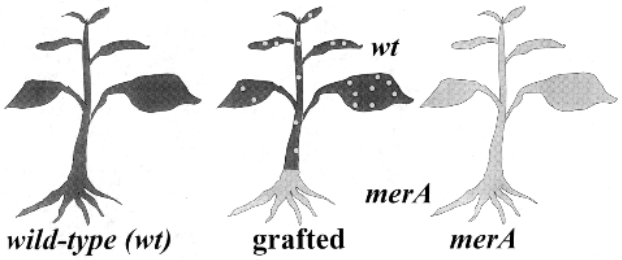
Summary of APGEN's Technologies

- Native plants degrade organics efficiently- should be no issues of public acceptance
- Heavy metals require transgenic technologies - focusing on worst heavy metal toxins now and radionucleides in the future

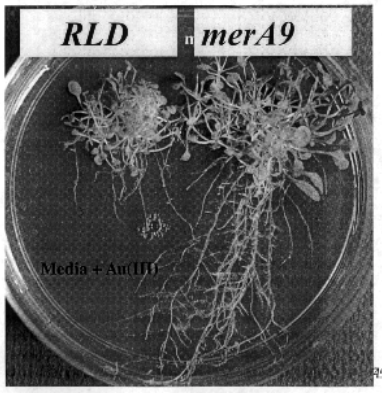
Planned Future Directions

- **Engineering above-ground hyperaccumulation**
- **Detoxification of other toxic heavy metal ions (cadmium, arsenic, copper, lead, & chromium)**
- **Phytomining of gold, silver, & platinum**
- **Genomics to identify thousands of relevant plant genes for enhancing phytoremediation**

Engineering a hyperaccumulator:
Wild-type shoots accumulate 10x more Hg(II) when grafted with *merA* roots



Phytomining: The expression of *merA* confers gold ion (Au(III)) resistance to plants



Special Concerns for Peconic River Site #1

- Only native plant species used for organics
- Wetlands viewed as a productive resource
- APGENs phytoremediation looks at most active species not just any species
- Native trees, herbaceous plants, and sterile grasses for transgenics

Special Concerns for Peconic River Site #2

- Have technology for Hg and DDD in place
- Preliminary research on Ag and Cu.
- Treatability study could identify PCB degrading plants in 4-6 months
- Hg research supported by DOE EMSP
- We're linked with CRADA for Peconic Site
  - Collaboration with Mark Furhmann (BNL), a phytoremediation & metals expert